

SEX-RATIOS AND SPERMATO-
GENESIS IN THE TOP-MINNOW,
GAMBUSIA HOLBROOKI GRD.

DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE
JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

BY

SAMUEL WOOD GEISER

Baltimore, 1922

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(From the Zoölogical Laboratory of the Johns Hopkins University.)

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I. INTRODUCTION.

Sex-Ratios Reported in Gambusia and other Pœciliids.—Many observations have been recorded of the sex-ratios in field collections of *Gambusia* and other viviparous members of the family

TABLE I.
REPORTED SEX-RATIOS FOR PŒCILID FISHES.

Species.	Locality.	Ratio $\sigma : 100 \sigma$	No. of Indiv.	Remarks.	Authority.
<i>Cnesterodon to-maculatus</i>	Central and South America	150	5		Henn, 1916
<i>Diphyacanthia chocoensis</i>	" "	357	32		" "
<i>Filzroya lineata</i>	" "	600	7		" "
" <i>maculata</i>	" "	533	23		Smith, 1892
<i>Gambusia affinis</i> (= <i>holbrooki</i>)	Lower Potomac River	High!	69	"1 male to 68 females"	Smith, 1912
" "	" "		95 +	"Several males and abt. 90 females"	
" "	Pasquotank R., N. C.	300	—	"One out of four males"	Smith, 1893
" "	North Carolina	High!		"Males only 2%—3% of a population"	Smith, 1907
" "	" "	High!		"Males very rare and not often collected"	Jord. & Ev., 1896
" "	" "	800—900			Hildebrand, 1917
" (= <i>affinis</i>).....	Mound, La.	187—	Many!		Barn. & Ans., 1921
" (= <i>holbrooki</i>)	Beaufort, N. C.	882			Geiser, 1921
" <i>punctata</i>	W. Cuba	257	693		" "
" <i>puncticulata</i>	W. Cuba	775	796		Eigenmann, 1904
<i>Glaridichthys unimolatus</i>	W. Cuba	High!			" "
<i>Jenynsia lineata</i>	" "	High!			Weyenbergh, 1863
" "	" "	500			Thumm, 1908
" "	" "	Low!			Boulenger, 1912
<i>Lebistes reticulatus</i>	" "	100—250		"Freq. purely ♀ litters one after another"	
" "	" "			Diff. survival rates at diff. temps.	
" "	Barbadoes	100		With diff. sex-ratios of litters	Henn, 1916
" "	" "	116	Many!!	"Normally a 1 : 1 ratio"	Schmidt, 1920

TABLE I (continued).

Species.	Locality.	Ratio ♂ ♀ : 100 ♂.	No. of Indiv.	Remarks.	Authority.
<i>Leptorhaphis infans</i>	Mexico	High!	78	"Fewer males than females"	Meek, 1902
<i>Limia vittata</i>	W. Cuba	156 200	82		Eigenmann, 1904
<i>Limia hollandi</i>	Central and South America	1333	105		"
<i>Mollienisia formosa</i>	*	High!!		"Purely fem. litters one after another."	Henn, 1916 Thumm, 1908
<i>Mollienisia latipinna</i>		78	71		Everm. & Goldsb, 1902
" <i>occidentalis</i>	Rio Yaqui, Sonora	267	16		Ruttier, 1896
" <i>spilurus</i>		High!!!	33		Stansch, 1911
"		High!!		"Progeny almost all females"	Milewski, 1920
<i>Neoheterandria elegans</i>	Colombia	300			Henn, 1916
<i>Phalloceros caudomaculatus</i>	S. Amer.	334	16		Henn, 1916
<i>Phallopitychus eigenmanni</i>	Bahia, S. Amer.	450	262		Henn, 1916
<i>Platypocilus maculatus</i>	*	148	11		Bellamy, 1922
<i>Poecilia parae</i>	British Guiana	136	669		Eigenmann, 1909
" <i>vivipara</i>	Brazil	362	38		Henn, 1916
<i>Pæciliopsis presidionis</i>	Colombia	656	37		Eigenmann, 1912b
<i>Priapichthys episcopi</i>	Canal Zone	400	121		Everm. & Goldsb.
"	"	16	5		1909 and 1910
" <i>nigroventralis</i>	Colombia	900	29		Eigenmann, 1912b
<i>Pseudorhaphophorus</i> Sp.....	Central and South America	110	10		Henn, 1916
" <i>bimaculatus</i>	Mexico	127	21		Jord. & Everm., 1896
"	*	High!	34	"Nearly all ind. in 2 litt. males"	Langer, 1913
<i>Tomeurus gracilis</i>	British Guiana	Low!!			Eigenmann, 1909
<i>Xiphophorus helleri</i>	"	100	6		Bellamy, 1922
"	"	68	317		

* Aquarium populations.

of the top-minnows or *Poeciliidæ*. The results in reference to sex obtained in these collections are presented in Table I. By referring to this Table it will be seen that in practically all ratios reported for *Gambusia*, and in 80 per cent. of the records of the poeciliids generally, the females were greatly in excess.

It has been suggested that the discrepancy in the proportions of the sexes is due to the fact that in this group of fishes the males are much smaller and more agile than the females, and hence either more readily avoid, or pass through the meshes of, the net. However, in those cases where the net used had a mesh so small that loss of the smaller males was thereby excluded, collections still showed the same inequalities of numbers of the sexes. It therefore seems well-established that in the field there is usually, if not always, a marked excess of females in these fishes. The writer has had populations under observation in which the proportion of males present was as low as 3.8 per cent. By the method employed in catching them it was not possible for a single individual, male or female, to escape.

Moreover, Hildebrand ('17) observed the sex-ratios of specimens of *Gambusia* raised in the laboratory under conditions in which every individual was examined, and found them to be approximately the same as those observed in collections taken in the field. He concludes ('17, p. 10) that "It seems entirely probable that the normal ratio of males to females is about 1 to 8 or 9."

Mast ('21) found on dissection that, in some populations of *Gambusia* that he studied, a considerable proportion of the individuals which looked like females and had been counted as such were really males in which the secondary sex-characters were not developed. He designated these "sterile males" and suggested that the reports of excessive numbers of females might be due to the fact that many such males might be counted as females. However, in the course of the writer's studies, when large numbers of "females" from populations showing low percentages of males were dissected to ascertain whether a considerable number of these "females" might not really be sterile males it was found that the percentages of sterile males in such populations was too small to account, even in a small

degree, for the very great disproportion in the numbers of the sexes.¹

It must therefore be concluded that the inequality of the sexes of adult *Gambusia* is real, and cannot be accounted for on the basis of faulty methods of collection or of inability to recognize the sexes. This fact has led some investigators to hold that sex in *Gambusia* is conditioned by obscure fluctuating environmental or physiological factors, and that it is not unalterably determined at the time of fertilization. Conclusions similar to this have been held by Woltereck ('08) in reference to various *Poeciliids*, and by Okkelberg ('21) in reference to the brook-lamprey.

Sex-Ratios in Fishes.—But little work has been done on the sex-ratios of fishes generally, and nearly all of this only on populations not under experimental conditions. In this work the enumeration was only of adults, usually by superficial examination only, and hence the value of the findings is very uncertain in the presence of so many variables. That is, the sex-ratios reported for fishes have in nearly all cases been simply "sex ratios of collection," and explicable upon a number of hypotheses. Darwin ('75) and Fulton in various papers, have largely given us what little data we have on the subject.

Among the factors that might cause atypical ratios in adult fish two alternative ones occur most readily to the mind: (a) a possible differential death-rate of the sexes during the embryonic, juvenile, and adult period, coupled with a normal sex-ratio at fertilization, and (b) an atypical primary sex-ratio, due to an atypical distribution of sex-determining chromosomes to the two daughter cells in the maturation divisions of the germ-cells. As a result of this unequal distribution of the sex-chromosomes, a preponderance of one sex over another could conceivably be produced. The approximate proportions found

¹ Essenberg (BIOL. BULL., Vol. 45, pp. 46-97, 1923), in his very interesting paper finds that in old populations of *Xiphophorus* the percentage of males is often very high. He concludes on the basis of his observations that in this species there may be large numbers of "retrogressive females" which later transform into males. In my observations on *Gambusia* I have never found intersexes or instances of sex-inversion.

(one male to three or seven females) are suggestive of this explanation of the atypical adult ratio.

The special chromosomes whose presence or absence appears in many animals to be associated with a definite sex have not yet been reported² on cytological grounds for any teleost fish. If we knew that such a chromosome (or chromosomes) existed in the sex-cells of a given species, thus giving rise to dimorphic zygotes on fertilization, we could forecast approximately equal numbers of the sexes at birth (secondary sex-ratio of Schultz, '18), unless the gametes showed a differential viability. This cytological method of ascertaining primary and secondary sex-ratios is of course the most dependable one we have. Failing in this, valid conclusions may also be reached by taking large litters and raising them with as few mortalities as possible to a stage where the sexes may be distinguished. Unless the early sex-ratios are ascertained by one of these methods, the results are quite untrustworthy. Apparently only three workers, Eigenmann ('96), Punnett ('04), and Hubbs ('21) have done this. The first mentioned, in his work on the viviparous Embiotocid *Cymatogaster* ascertained the sex-ratio of litters before birth by microscopical examination of the embryo-gonads, and found the sexes approximately equal in numbers in the litters. Punnett ('04) in a similar way found the sex-ratio in the Elasmobranch *Spinax* to be approximately a 1:1 ratio, and Hubbs ('21) obtained results similar to those of Eigenmann in the Embiotocid teleost, *Amphigonopterus*. Only the sex-ratios of adults can be learned by external observation of the individuals of a population. It is certain, of course, that if no differential death-rate of the sexes exists in a given species, the sex-ratios of adults will give a trustworthy index to the sex-ratios at fertilization and at birth, as appears to be the case in the lake whitefish, *Coregonus albus*, as reported by Pearl ('16).

As has been said, in *Gambusia* the females are greatly in excess of the males. The following cytological and experimental studies were undertaken with the view of ascertaining whether

² No differences have been found between the sex chromosomes and the autosomes in the poeciliid teleost, *Lebistes*. (Winge, '22a, also in C. R. Lab. Carlsberg, Vol. 14, No. 17, p. 8, 1922.)

this is owing to peculiarities in the distribution of possible sex-determining chromosomes, or to a differential viability in the gametes, or the zygotes, both in embryonic and juvenile stages.

II. SPERMATOGENESIS IN *Gambusia*, WITH SPECIAL REFERENCE TO ITS BEARING ON THE EXPLANATION OF THE SEX-RATIO.

The illuminating genetic studies of Schmidt ('20) have demonstrated that *Lebistes* has an XX, XY constitution. Aida ('21) has also shown the same thing to be true of another Poeciliid fish, *Aplocheilus*. Since *Gambusia* and *Lebistes* are closely related it is reasonable to assume that the chromosomal arrangement in these two forms is similar. If this is true, then we should expect in *Gambusia* equal numbers of potential males and females at the time of fertilization, and if this is what actually obtains, then the preponderance of females observed in adult populations must be due to a differential death-rate operating after fertilization. The importance of the investigation of spermatogenesis in this form is consequently obvious.

But little work has been done on the spermatogenesis of teleost fishes. The only title that the writer has been able to find is that by Turner ('19) on the seasonal cycle of the testis of the perch.³ Bohm ('91) and Behrens ('93) have investigated the oögenesis of the "Forrelle" and Blanc ('94) has studied the oögenesis of *Trutta lacustris*. Mrs. Harvey ('20) in her synoptic paper has noted brief observations by other authors on diploid cleavage numbers in a number of other teleost fishes. But except for the paper of Turner above noted no study appears to have been made of the male sex-cells of teleosts. The explanation seems to lie in the fact that in this group of fishes the chromosomes are very numerous, small in size, and lack individuality.⁴

³ Since the above was written, Winge ('22a) has briefly described the spermatogenesis and oogenesis of *Lebistes reticulatus*, and Essenberg ('23) has published brief observations on spermatogenesis in *Xiphophorus*, a poeciliid fish.

⁴ In *Lebistes* and *Xiphophorus* the same difficulty is found. (Cf. Winge, '22a, and Essenberg, '23.)

Materials.—The *Gambusia* used in this study were from the P₁-stock of the experimental litters (*q. v.* in Section III. of this paper). Thus the material for both cytological and breeding studies was from the same source. The stock had a very great preponderance of females, the males being outnumbered by the females nearly eight to one.

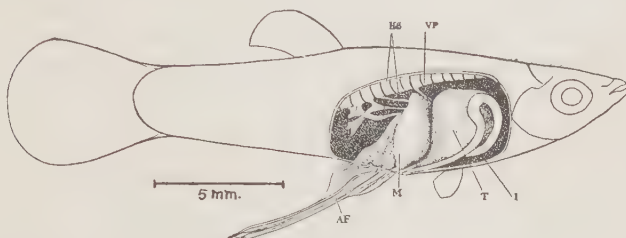
Methods.—Preliminary tests with Flemming's solution, and with many modifications of Bouin's fluid, particularly Allen's ('18) modification, demonstrated the fact that the best fixation of prophase stages in the material was obtained by the use of Bouin's fluid, modified by the addition of one per cent. of Merck's *c. p.* urea crystals. This was the fixing agent employed in all subsequent work on the cytology of *Gambusia*. With the best of care, however, it was not possible with any fixing solution to get good separation of the chromosomes in the spermatocyte metaphase plates, even though the earlier phases in the same section were beautifully fixed. This difficulty in working with teleost testes is one that has troubled a number of workers. Turner ('19) in his work on the perch, *e.g.*, was unable to obtain spread equatorial plates of chromosomes.

Both section and smear preparations were made. Sections were cut five and seven micra thick. All preparations were stained by Dodd's ('10) long process, with iron-hematoxylin. Occasionally counter-stains were used.

The General Anatomy of the Testis.—In the adult fish the two testes are fused, forming a single gland lying just below the posterior portion of the swim-bladder, and anterior to the gonopod (Text-fig. 1). The posterior portion of the intestine lies below and anteriorly to the combined testes (hereafter called the "testis"). The large muscle controlling the movement of the gonopod lies just behind the testis.

The testis is whitish in color. It lacks the heavily pigmented investing membrane so characteristic of the ovary. It is suspended from the swim-bladder and the vertebral column by a very thin mesorchium. The surface of the testis when examined in a fresh condition shows a more or less favose network of cell-borders under the thin investing membrane. These nettings are caused by the arrangement of the cysts in the testis.

The testes vary in size with the season and the individual. Thus, the median length of 19 taken at random on July 20, 1920 was 3.2 mm. and the median breadth 2.2 mm.; while the median length of five taken November 23, 1920 was 2.75 mm. and the median breadth 2.07 mm., a volume relation of approximately 1.6 to 1. The testes in individuals of the same litters vary but little in size, but they vary greatly in individuals of the same size taken from different litters.

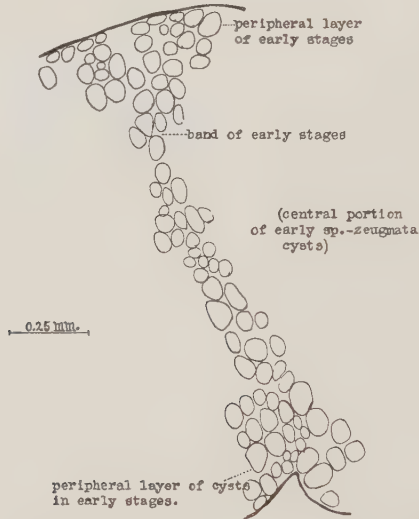


TEXT-FIGURE 1. Lateral dissection of a male *Gambusia holbrooki* showing the general relations of gonopod to other organs, especially the internal organs of the body. *I*, intestine; *T*, testis; *AF*, gonopod; *HS*, modified haemal spines; *M*, muscle controlling gonopod; *VP*, ventral process of abdominal vertebrae. (After Kuntz, '14.)

There is no prominent connective-tissue core in the testis of *Gambusia* such as Turner ('19) described for the perch, and Brock ('78, Taf. xxviii, Fig. 3) figured for *Perca fluviatilis*. The testes of *Gambusia* consist of a tenuous, diffuse, connective tissue stroma, in the interstices of which the spermatogonia and cysts are closely packed. The structure of the adult testis resembles very closely in some respects that observed by von Ihering ('83, p. 486) in the South American poeciliid, *Phalloceros caudomaculatus*. The smaller cysts of multiplying spermatogonia lie in the cortical portion of the testis. As one proceeds inward the cysts become more and more mature. The cysts in the spermatid stage are much larger than the mature sperm-balls, or spermatozeugmata. The latter lie closest to the center of the testis, and near to (and with) the longitudinal canal, which appears to serve as a storage place for sperm; the former, more peripherad. After the growth-period preceding the I.-spermatocyte division, the cysts are much larger than

before. von Ihering's description of the formation of a spermatozeugma in *Phalloceros* holds also for *Gambusia*.

The testis of *Gambusia* lacks the tubular type of structure characteristic of the testes of oviparous Pœciliids, e.g. *Fundulus*. Apparently there has come with viviparity in this family fertilization of the eggs of the female by means of sperm-balls, which appear to be formed in the same way in all viviparous members of the family Pœciliidæ examined.



TEXT-FIGURE 2. A portion of the testis of *Gambusia*, showing the differential development of spermatocytes indicative of the original paired condition of the testis.

In the adults the two testes are very closely fused; the double structure being evidenced only by the almost universal separation of the longitudinal testicular canal into two portions, one ramus on either side of the median line in the anterior four-fifths of the testis; and in the differential development of spermatocytes (Text-fig. 2).

In the testis figured the major portion of the gonad consisted of cysts in spermatid and early spermatozeugma-stages. These occupied the central portion of the testis, grouped about the paired longitudinal testicular canals. At the periphery there were cysts whose cells were in early I.-spermatocyte division

prophases. Along the median plane there also occurred a band of cysts whose cells were in the same stage of mitotic division as those of the cysts occurring at the periphery of the testis. The line of fusion of the two original testes appears clearly in the vertical band of younger cysts running through the section figured.⁵

Seasonal Variation in the Adult Testis.—The testis shows a marked seasonal periodicity in volume, the number of contained spermatozuigmata, and the volume of the longitudinal testicular canal. During the spring and summer, which is the breeding season, the testis increases in size. The increase is, however, not large, as it averages only 60 per cent., while the maximum is only a sixfold volume-increase. This is much less than has been found for other fishes, e.g., the perch.

During the late autumn and winter and very early spring the testis is filled with spermatozuigmata which greatly distend the longitudinal testicular canal. After the great spring wave of copulation the testes show very few spermatozuigmata, and in June and July the testes are filled with early stages of spermatogenesis. Smears of the testes taken in August and September show only spermatids and spermatozuigmata along with late spermiogenesis stages.

The Spermatozuigmata.—When ripe, the spermatozuigmata are spheroid to ovoid bodies with a diameter in winter testes of 210-320 micra. They lack completely the investing membrane characteristic of spermatophores and agree entirely in structure with those described for *Cnesterodon 10-maculatus* by Philippi ('08.) It is probable that the error into which Kuntz ('14) has fallen with regard to the structure of the "spermatophore" is due to his having studied and described cysts containing immature spermatozoa. For in mature cysts and spermatozuigmata the spermatozoa lie at the periphery and have a definite arrangement.

Observations on Spermatogenesis. (a). Primary and Secondary Spermatogonia.—The definitive sex-cells by repeated divisions give rise to the final spermatogonia. It is not known how

⁵ Essenberg's "Figure 38" also shows very well the similar bilaterality of the testis in the related species, *Xiphophorus helleri*.

many fissions take place before the spermatogonia are produced which give rise to the final spermatogonial cyst. A computation based on the approximate total number of cysts produced during the lifetime of a male *Gambusia* gave the number of fissions as at least 16. Such a computation is to be accepted with reservations: certain it is, however, that there are many cell-divisions from the time of the first segregation in the Keimbahn to the time when the cyst-forming spermatogonia are finally located in the testis.

The time of segregation of the first sex-cells is not known. My earliest *Gambusia* embryos have a snout-anus length of 1.58-2.05 mm. Definitive sex-cells are then present in the typical location beneath the swim-bladder. At birth the gonads are developed but slightly beyond the condition found in these embryos. The sexes are not readily distinguishable. The gonads at birth have large, clear cells with a very clear nucleoplasm in which lies a prominent plasmosome. From the plasmosome, and apparently taking their origin from it radiate delicate linin fibrils, upon which frequent granules of deeply staining chromatic material may be seen. The filaments appear to extend to the inner surface of the nuclear wall. Occasionally the sex-cells in the gonad appear especially large and clear, and each cell is invested with a layer of mesoderm cells, but these have been observed in only a few individuals. It is not improbable that such cells are developing oocytes, but the evidence in the present case is not completely convincing. In a few of the individuals cells unmistakably developing ova are found at birth. These cases are however, rare. In those cells which have not yet differentiated there is usually a prominent plasmosome, with one or more other conspicuous irregular masses of chromatic material. In the later spermatogonia (Figs. 1-2)⁶ which are produced from localized peripheral germ-cells of the testis, in the cyst during its formation, there are usually two prominent karyosomes. The final spermatogonia (Figs. 8-10) are approximately one-eighth the volume of the cell which by dividing formed the cyst. These final spermatogonia possess a very fine

⁶ "Figures" refer to the plates.

reticulum of inconspicuous fibrils; the chromatin flakes are small and seen only with difficulty.

The early Spermatogonial chromosomes in multiplication-divisions in the cyst (Figs. 3-5) are simple rod-shaped elements, with apparently terminal spindle-fiber attachment. In no case was a J-, U-, or V-shaped chromosome observed in a polar view of the plates. The chromosomes in the spermatogonial metaphase plates are usually so numerous and so densely massed that a dependable count is almost precluded. The number, however, is not less than thirty nor more than thirty-six; in a single widely-spread plate 36 chromosomes were clearly seen. That this is the diploid number is suggested by the fact that in the diakinesis stage preceding the first spermatocyte division, counts of the tetrads give 15 and 18 as the number of quadrivalents. The diploid number of chromosomes in the male germ-cells would then be 36 if no heterochromosome were present, or 35 if one heterochromosome were present. On the latter point it will not be possible to speak definitely, since the II.-Spermatocyte plates are always too crowded and dense to show the exact number of chromosomes. It is apparent, however, that approximately 18 univalent chromosomes are present. Lagging chromosomes are frequently seen in anaphase stages viewed from the side, but not constantly enough to give a clue as to the number, size and form of possible heterochromosomes. Occasionally precocious chromosomes appear in early maturation-stages. If one were to trust the uncertain help of analogy, however, it might be claimed that the male *Gambusia* has one *heterochromosome*. (Cf. Schmidt, '20.) With this assumption, it would be quite within the bounds of probability that the spermatogonial chromosome number is really 35, the plate which showed 36 merely possessing a lagging chromosome belonging to the other daughter cell.

By repeated mitotic divisions the cysts become filled with the final spermatogonia (Figs. 8-10). The precise number of fissions of the spermatogonium forming a cyst is not known: computations indicate that at least 10, and often 12 fissions occur before the cysts are ready for spermatogenesis. In a given cyst the cells are all in the same stage of maturation and thus, as in

insect testes, it is possible to find all stages of the process, each in a different phase, in a single section.

(b) *Later Stages in Maturation. Leptotene. Synapsis. Pachytene.*—In the further maturation of the male germ-cells of *Gambusia* the process follows the usual path. There are no features worthy of special note. The filamentous chromosomes of the leptotene (Figs. 11-16) are produced by a condensation of the chromatin on the linin fibrils. There has not been any striking evidence of pairing of the chromosomes in the leptotene, owing largely to the difficulty of the material. In the synaptene and pachytene stages (Figs. 17-18) which follow upon the leptotene there is evidence that parasynapsis occurs. The chromatin threads in early synapsis become only half as numerous as in the leptotene. The pachytene shows the typical orientation of the thickened and shortened threads of this stage. No contraction-stages (*synizesis*) appear to occur in this species.

(c) *Tetrads and Diakinesis.*—The tetrads in their early stages appear to be of the open-ring type (Figs. 20-21). They contract however, as they take on the peripheral position characteristic of diakinesis (Fig. 22) until all trace of their tetrad character is lost. They then appear as large, spherical, deeply-stained bodies evenly distributed through the plasm just beneath the nuclear surface. In this position they remain for some little time, after which they gather to form an equatorial plate (Fig. 23).

The tetrads are halved in the I.-Spermatocyte metaphase. The distribution of the dyads to the two daughter cells of the first spermatocyte division could not be made out (Figs. 24-26). The II.-Spermatocyte division follows immediately upon the conclusion of the first division, with no nuclear reconstitution. After the secondary spermatocyte division is completed the chromosomes clump together, the chromatin changes in reaction to stains and becomes scattered throughout the nucleus. After the resultant spermatid nucleus has been constituted, a rather long interkinesis occurs. This is followed by a stage in which the chromatin gathers at the periphery of the nucleus and stains deeply.

(d) *Spermatozoa*.—The spermatids lose their cytoplasm; the nucleus becomes hemispherical (Fig. 31) then elongate (Fig. 32-33). As the spermatozoa come to nearly attain their mature form (Fig. 34) they migrate to the periphery of the cyst. The heads are directed outwardly, and the tails, together with a colloidal substance, fill the central cavity, precisely as shown by Philippi ('07) for *Phalloceros caudomaculatus*. These spermatozeugmata appear to retain their individuality until their discharge from the genital pore of the male. The first spermatozoa are formed in May and June, and the process continues until October. Their formation, then, is contemporaneous with the period of warm weather.

(e) *Dimorphism of the Spermatozoa*.—Zeleny & Faust in 1912 made the discovery that in certain insects, the males of which are heterozygotic, the ripe spermatozoa can be divided into two groups which differ considerably from each other in size, those in each group varying about a mode with but very little overlapping. Later work by these investigators (Zeleny & Faust, '15a, '15b) extended their early results to several groups of insects, and Wodsedalek ('13, '20) found the same dimorphism in the spermatozoa of mammals. In *Gambusia*, the writer was not able to find any such dimorphism. It is possible that when spermatozoa from different spermatozeugmata are measured, and the measurements grouped together, as was done in the present case, a complicating factor enters in the fact that the sperm-heads may thus be in different stages of maturity, and hence of diverse volumes. In view of the facts, however, that the sex-chromosome, if it occurs, is not very different in size from the other chromosomes of the cell, and that the chromosome number is so large, thus making the relative increase in size of the gamete possessing the sex-chromosome slight, it is doubtful if such measurements of sperm heads would furnish any clue to the existence of a sex-chromosome. Whether such a chromosome exists or not may be much more easily ascertained by breeding experiments involving sex-linked characters, as e.g., Schmidt's work, already referred to.

From the results obtained by the study of the spermatogenesis of *Gambusia*, no light is cast on the origin of the anomalous sex-

ratio of adults. The cytological evidence, while not satisfactory, points to an apparently nearly equivalent division of the chromosomes. The presence of heterochromosomes is indicated. The cytological condition of related forms would seem to indicate the production of equal numbers of male and female-determining gametes. What the proportions of the sexes in experimental litters with known life-conditions would be, remains to be ascertained.

(f) *Summary on Spermatogenesis.*—The testis of adult *Gambusia* is formed by the median fusion of two original paired testes. This fusion takes place within the first two months of independent life. Traces of the double character of the adult testis are shown in the usual division of the longitudinal testicular canal, and in differential maturation along the median plane (line of fusion). The testes in summer are somewhat larger than in winter. The relative proportions vary markedly with diverse individuals. The fluctuation in volume is not so great as in other teleosts.

The testis lacks any strongly-developed connective tissue core. There are no spermatic tubules. The cysts of spermatozoa are the result of the continued fission of germ-cells which at the season of sexual activity migrate to the periphery of the gonad.

The gonads at birth are undifferentiated, but sex is ascertainable within four weeks after birth, food and other conditions being optimum. The period of active sexual life is the spring and summer months. Active spermatogenesis occurs only at this time.

There are no special features of spermatogenesis in *Gambusia*. The cysts are produced by 10-12 fissions of an original spermatogonium. The final spermatogonia pass through the usual prophase-stages. Each cyst contains approximately 1500-3250 final spermatogonia, all in the same stage of development. During spermatogenesis, this relation continues.

It appears from counts of spermatogonial metaphase plates and from diakinesis stages that the diploid number of chromosomes in *Gambusia holbrooki* is 35 or 36. There is no evidence in the spermatogenetic process of an unusual method of chromatin

distribution that would explain the occurrence of the atypical sex-ratio observed in the adults.

III. OBSERVATIONS ON SEX IN *Gambusia* RAISED IN AQUARIA WITH SPECIAL REFERENCE TO ITS BEARING ON THE PROBLEM OF SEX-RATIOS.

Observations on the Sex-Ratios of the Aquarium Populations.—During the years 1919-1920 a series of experiments with gravid *Gambusia* was conducted with a view to ascertaining whether it were possible in these fish to alter the sex-ratios of the young at birth by the feeding of male gonads to the pregnant females. It was known that the males and females cannot be distinguished by superficial observation, but it was assumed that they could be distinguished by cytological methods. Consequently the young of the experimental females were taken at birth, or several days later, fixed in Bouin's fluid, and the entire animal later sectioned and studied. It was found on studying the sections thus obtained that while in some of the individuals unmistakably definitive oocytes occurred in the usual position just beneath the air-bladder, in others the gonads were still undifferentiated. No internal structures, such as genital ducts or other features gave an indication of the sex. It was consequently evident that the sex of *Gambusia* at birth cannot be ascertained by cytological methods.⁷

The next endeavor was to raise the young in laboratory aquaria until the sexes were distinguishable. At birth they cannot be so distinguished. In adults it can be done by superficial examination of the anal fin, or by dissection. At the age of three weeks after birth it can be done by the cytological method, and at the age of three months, if life-conditions are optimum, by the examination of the anal fin.

⁷ In this connection, it is interesting to note that since the above was written, Champy & Gley (*Arch. d'Anat. microscopique*, T. 19, Fasc. 2, p. 259, 1923) have found with the poeciliids *Haplochilus*, *Poecilia*, *Lebistes*, *Xiphophorus*, and *Platyphacilus* a very tardy development of the ovary of the young fish. They note that the state of maturity of the gonads is not attained until the animal has acquired its adult size, which is here sharply defined, and that the gland remains in an immature state in animals almost as large as the average-sized sexually mature fish.

These experiments endeavoring to raise the young in laboratory aquaria until the sexes were distinguishable came to naught, for the mortality in indoor aquaria was so great that even with the best of care and feeding the differentiation of the sex-glands did not occur during the maximum period that it was possible to keep any considerable portion of the litters alive. Microscopic study of those individuals which had died during the experiments cast no light on the proportions of the sexes of these fish.

Before proceeding further it may be well to consider the criteria of sex in young and adult *Gambusia*.

Criteria of Sex in Young and Adult Gambusia.—The viviparous Pöciliidæ, such as *Gambusia*, possess a well-marked sexual dimorphism. The males are much shorter and more slender than the females. The anal fin in the male is placed farther forward than in the female, and is modified into a conspicuous "intromittent" or copulatory organ, the *gonopod*. This dimorphism of the sexes is so marked that in one species male and female were originally classified in different genera, and their identity was disclosed only after a lapse of years, when individuals of the assumed two genera were observed to copulate. The sexual dimorphism of the adult constitutes a very serviceable criterion of sex, but in juvenile, immature forms it is inadequate. Studies to ascertain the limits of variation of sex-characteristics, such as position of the anal fin in male and female, were therefore made by the writer in order to gain data which would permit one to ascertain readily, without dissection, the sex of an individual suspected of being a "sterile male." A study was also made of the development of the anal fin in the male from the indifferent stage to that of a fully-developed gonopod, for data concerning the time of first appearance of the gonopod, the most striking characteristic of the male, and its degree of development and differentiation in early stages were considered of the greatest interest and importance. A reliable criterion of sex in the early period of life of the fish was much desired.

The Gonopod and its Development.—The modified anal fin in the various groups of Pöciliids becomes a gonopod in accord-

ance with a definite structural plan. The gonopod is formed by the modification of the third, fourth, and fifth anal-fin rays in the species whose anal fin possesses from six to ten rays. The general plan of the gonopod, with few exceptions, is as follows. The first and second ray are undivided and very short; the third ray is very much elongated; the fourth and fifth, also, are considerably elongated; while the sixth to the tenth rays are normal, bifurcate, and segmented. The third to fifth finrays, also, possess modified ossicles. (See Geiser, '23b.) Genera and species, while following this general foundation-plan, vary widely in the details of structure of the gonopod, as Langer ('13) has so beautifully shown. The primitive form of the gonopod occurs in *Petalosoma*. The apex of the gonopod may have a spoon-shaped process, the "prepuce" to aid in the transference of the sperm-balls to the genital papilla of the female during copulation. Such is the case in *Petalosoma*, *Pæcilia*, and *Molliensis*. Other modifications serving the same purpose are found in *Phalloceros*, in the form of hooks; and in *Phallotorynus* in the form of a curious structure resembling a garden trowel.

The writer's observations on the finer structure and progressive differentiation of the gonopod itself are reserved for future papers.

Hildebrand ('17) has called attention to the fact, also, that in young *Gambusia* the anal fins of the males and females are similar, and that the gonopod develops gradually, and at no definite age or length of the fish. When the young are only 13 mm. long and less than three months old the gonopod is sometimes developed: on the other hand, the fish may be 17 mm. long and five months or even a year old, and it is still not developed. A lot of 43 young of which he writes, born in May, 1914, were examined on October 15 of that year (when the smallest was 17 mm. long) and it was thought that they all were females, as no gonopod had developed. On June 3, 1915, however, six of the surviving fish were easily recognizable as males. The mortality was not stated, although he remarks elsewhere in his paper that the death-rate during early life in aquaria is inordinately high. From his results he concluded that the "modification of the anal fin into an intromittent organ may take place when the

fish reaches a length of 13 mm., or at any later stage until it attains its maximum normal growth of about 25 mm."

Mast ('21) as noted above, has given further evidence of the tardy development of the gonopod in the "sterile males" of *Gambusia holbrooki*. He found in these sterile males that the testes had already differentiated and were in a condition of advanced spermatogenesis. Even ripe spermatozoa were present in the form of sperm-balls. Yet with all this evidence of functional maturity, the anal fin gave no evidence of the sex of the individual.

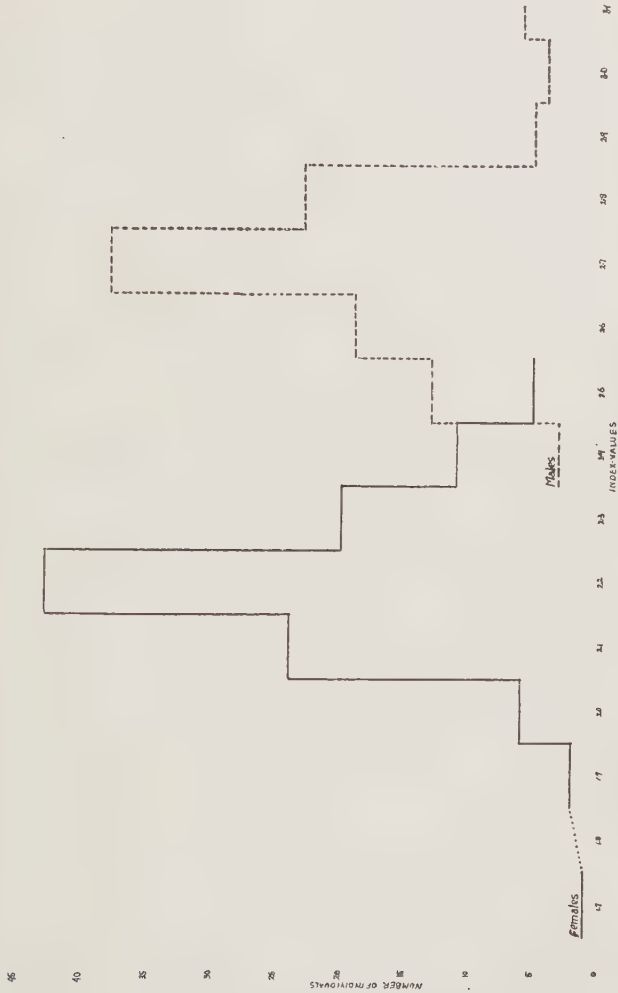
The writer's studies show that the gonopod develops rapidly during the summer, so that by the time the season is over practically all the gonopods are developed and recognizable as such. The differentiation of the terminal portion of the gonopod into the characteristic hooks of, especially, the third, and the posterior branch of the fourth fin-rays does not, however, take place often until very late in the Fall or during the following Spring.

It is clear that this is hardly a criterion of sex that can be serviceable with younger fish of this species.

The Position of the Anal Fin as a Criterion of Sex.—In *Gambusia* the anal fin migrates forward in the male during the period of sex-differentiation with the result that when adulthood is reached the relative positions of the fin in the two sexes is quite dissimilar. *The exact value of this "index," *i.e.*, the relation between the total length of the fish and the distance from the tip of the nose to the anterior border of the anal fin, is easily calculated. Measurements made of a large number of Pöciliid fish, embracing several genera, show that the adult index is always higher in the male, *i.e.*, the anal fin is placed relatively farther forward in the male. The mean index-value varies with the different species.

Graph I. shows the index values of two lots of *Gambusia* obtained from Beaufort, N. C., in 1920/21. While there is considerable variation, the indices for both sexes have very definite norms. As will be seen from the graph, in the males the class 2.7-2.799 is the mode; in the females, the class 2.1-2.199 is the mode. A comparison of the data shows that there is very little overlapping. Thus, in this population, only 3.6 per cent. of the

males have an index-value of less than 2.500; while on the other hand, only 4.3 per cent. of the females show an index-value in excess of 2.500. The findings in the populations graphed are paralleled by the results of other series of measurements.



GRAPH I. Showing the mutual exclusiveness of the Index-values of an unselected population of adult male and female *Gambusia*.

From this we see that the indexes for adult male *Gambusia* are such that by means of them the male can easily be distinguished from the females, in the lack of better criteria, even with rather old "sterile males" in which the gonopod is not well

developed. The indices have rather sharply-defined limits, and these are nearly mutually exclusive. Applying these facts to the examination of suspected male *Gambusia*, we find that the number of sterile males is inconsiderable and quite inadequate to explain the occurrence of unusual sex-ratios.

With young, immature *Gambusia*, however, the adequacy of this criterion of sex is extremely doubtful. In the case of young, if the gonopod has not commenced to differentiate, the only reliable method of ascertaining the sex of a given individual is by cytological examination.

After this digression on criteria of sex, we may resume discussion of methods.

Methods and Results.—During the spring of 1921 it was found possible in large indoor aquaria to raise young *Gambusia* and to keep them, with very little mortality until the males could be distinguished from the females. Four litters were raised as follows:

On May 15, 1921 four females, each approximately 45 mm. long, and well advanced in pregnancy were taken at random from a lot of gravid *Gambusia* which had been collected at Beaufort, N. C., March 22, and shipped to Baltimore. These were isolated in special breeding aquaria which consisted of ordinary 3.5-liter battery jars in which were hung cages made of wire netting of 3.5 mm. square mesh. The cages were coated with beeswax to prevent rusting. The gravid females were put into these wire baskets, one in each, and the aquaria stocked with plants as usual. When the young were born they darted out through the meshes of the mother's cage and were thus able to escape her cannibalism. *Gambusia* females very frequently eat their new-born young—a habit that is shared with some other viviparous cyprinodont fishes.

The females were fed a variety of foods: boiled white of egg, bread soaked in beaten egg and dried, and finely chopped snails. They devoured eagerly microcrustacea, enchytraeid worms, and mosquito larvea, but these food materials were not always available.

The four females designated *a*, *b*, *c*, and *d* had young on the following days: May 19, May 19, May 21, and May 21, re-

spectively. Female *a* had a litter of 37 young; *b*, of 19; *c*, of 22; and *d*, of 24. The birth of all these litters except that of *a* was observed, and it is known with certainty that none of the young were eaten by the mothers. It is most probable in the case of Female *a* that none were eaten, for in the writer's stocks, 37 is a very good litter. As soon as parturition was completed the mothers were removed. The young were fed on *Daphnia* for a few days in the small aquaria where they were born; after which time they were removed and put into concrete aquaria 90×160×60 cm. These pools had been thoroughly cleaned out in the spring and stocked with *Elodea* and *Spirogyra*. During the summer the *Elodea* grew until it formed a thick forest of vegetation in the aquaria, offering excellent protection to the young *Gambusia*. Litter *a* was put into one of these concrete aquaria, while Litters *b*, *c*, and *d* were combined and put into another. They were left in these aquaria without food, except such as came to them in the form of insects, etc., until the first group was 26 days old, and the second group three and a half months old. They were then removed, killed, and fixed in modified Bouin's fluid. All of the 37 young in Litter *a*, and 58 of the 65 of the other litters, were recovered. In the aquarium containing the latter there were found in addition to the 58 individuals, 12 which were relatively very small. These twelve were evidently offspring of some of the 65 individuals which had been put into the aquarium in May.

The proportions of the sexes in Litter *a* were learned by a histological study of the gonads of all the individuals. The young were taken after fixing was completed, the viscera dissected out in a mass, and these sectioned and studied. The gonads in all of these were differentiated to such an extent that the sexes could readily be ascertained by histological examination.

The sex of the young in the litters which were three and a half months old when killed could be readily be learned with certainty by examining the gonads under a binocular microscope. This was done in all of the specimens except those which had well-developed gonopods and were consequently unquestionably males. It was found that all the males in this lot had developed a gonopod, so that the presence or absence of the gonopod

would have been a satisfactory criterion of sex in the three and a half months old fish. However, to avoid any possible mistake, through the existence of sterile males, the gonads themselves were examined.

In Litter *a* there were 19 males, 17 females, and one whose sex could not be ascertained because a mistake in technique lost the gonads during imbedding. The individual whose sex is uncertain, however, was probably a female, as such a notation was made in the dissection notes. In Litters *b*, *c*, *d*, of the 58 surviving fish 27 were males and 31 were females. For the total group of 94 young *Gambusia* whose sex was learned with certainty, there were 46 males and 48 females, a very close approximation to a 1:1 ratio.

It will be recalled that in Litters *b*, *c*, and *d* seven fish were not recovered, and presumably died. If half of these dead were males and half were females, the proportions of the sexes would then show a still closer approximation to equality. Even if all the dead were females, still the sex-ratio would not even begin to approach the great disproportion found in adults.

In these populations, taken all together, the percentage of individuals whose sex was unaccounted for is very low—only 8.3 per cent.—so that the approximate equality of the sexes is not accountable for on the basis of a differential death-rate in favor of the males. On the other hand, there is evidence to show that the converse condition is responsible for the slight numerical inequality of the sexes in the older litters.

Corroboration of the conclusion reached that the numbers of males and females in *Gambusia* at birth are approximately equal is found in the results obtained by the examination of populations from two large pools kept under conditions as nearly ideal as possible. *I*. Into one of these pools there were put in May, 5 pregnant females. About the middle of October 45 young and three of the parents were recovered. Of these 45 young, 21 were males and 24 were females, again a very close approximation to equal numbers of the sexes. *II*. In a pond known locally as the "Euglena Pond" all of the *Gambusia* had died during the winter of 1920. This pond was stocked in May, 1921, with 48 gravid females. In the following October 284

individuals were taken with a dipnet at random from the pond. Of these 284 *Gambusia*, 94 were males, 60 were females, and 130 were so small that the sex could not be ascertained by external observation. Both of these collections were from the same parental stock as the females whose litters were studied. One is impelled to conclude as a result of these observations that the great excess of females in the parental stock (nearly 8 to 1) does not represent the proportions of the sexes at birth, but must have been due to a greater mortality of the males during either the juvenile period or later.

The only careful piece of work that has hitherto been done in the attempt to ascertain the sex-ratio at birth in *Gambusia* is that by Hildebrand, and his results are vitiated by a high death-rate. He found that five months after birth, of an original litter of 46 fish, none had developed a gonopod; and at the age of approximately 13 months, only six of the surviving fish possessed a gonopod, thus giving a very low ratio, even if a possible very high death-rate, which he mentions, is taken into consideration. But, as has already been seen, the factors of food and temperature are particularly potent in determining the rapidity of sexual development and the production of young in these viviparous fish. This was shown clearly in the work of Schmidt ('19, '19a),⁸ as well as by the writer's studies on duration of pregnancy and gonopod-development.

In confirmation of the correctness of the conclusion that the normal secondary sex-ratio in at least some Poeciliids is approximately 1:1, Henn's work ('16) may be brought forward. A total of 2,070 individuals of *Lebistes reticulatus*, the millions fish, was obtained in a single collection with a very fine-meshed net in the Barbadoes under the direction of Professor C. H. Eigenmann. The collection gave an approximately 1:1 ratio. In the lot there were 520 males and 630 females, besides 920 fish less than 10 mm. long, and too small to permit ascertainment of the sex by external examination. Henn says on the point of the sex-ratio that "it is quite certain that this count of males includes only members of that sex while a few of the smaller speci-

⁸ Duration of gravidity in *Lebistes* at 25° is about one month; at 18°, more than three months. (Schmidt, '19b, p. 4.)

mens regarded as females may really have been immature males. It will thus be seen that the sex-ratio, when an adequate collection is at hand, does not materially differ from that found in other fishes." He applies this conclusion to the *Poeciliidæ* generally. Schmidt's ('20) experimental litters of *Lebistes* had a ratio of males to females of 100:116.6 (total of 78 young) and his other data also show an approximate equality of the sexes. *Lebistes* possesses very many physiological and cytological characteristics in common with its close relative, *Gambusia*. It would be a singular thing if certain closely-related species of a compact family such as the *Poeciliidæ* possessed an anomalous ratio of the sexes at birth, as has been assumed for *Gambusia*.

In a very recent paper Aida has given data which demonstrate a practically even sex-ratio in a Japanese fresh-water poeciliid fish, *Aplocheilichthys latipes*. On assembling his data for His "Experiments 7-10, 10A and B, 11A and B, 12, 14, 17-21" (all data given) we find that he had a total in his experimental litters of 2,438 females and 2,400 males, a sex-ratio of 100 males to 103.9 females. This is very close to unity.

The Chromosomal Constitution of the Poeciliidæ and its Bearing on the Sex-Ratio.—It is unfortunate that the chromosomes of teleosts are so unsuited to investigation because of their small size, lack of individuality, and tendency to clump on the equatorial plate. It may be proper however, to consider in detail in this connection some genetical investigations whose results give strong evidence of the type of genetical constitution possessed by *Poeciliids*. Schmidt ('20) in breeding-studies was able to isolate a color-marking in a race of *Lebistes* which was transmitted through the Y-chromosomes. He undertook crossing experiments with two types of males on one type of females. The "new" male type, which we will call "B," possessed a brilliant dorsal fin-spot which was entirely lacking in the "old" type, "A." Other color characters made the two types instantly distinguishable. Crosses were made of Type A♂ Type B♀ and Type B♂ Type A♀, and back-crosses were made with F₁ populations. Breeding records were kept, with copious notes (and frequently water-color drawings) of a large number of individuals (*e.g.*, the registered males of F₂-F₆ total 998.) It was found

as a result of all these crosses that the dorsal fin-spot of Type B was carried through the male parent only. This fact was demonstrated very beautifully by Schmidt's experiments, and caused him to conclude that the genetic constitution of this fish is of the XX, XY type, and that the Y-chromosome carries the factor for fin-spot. Since this paper was written, Winge has (22a, 22b) further demonstrated both cytologically and genetically the XX, XY constitution of the Pœciliids, and has greatly extended Schmidt's genetical studies. Aida ('21) has shown by his breeding experiments, also, that another Pœciliid, *Aplocheilus*, also has an XX, XY genetic constitution.

If, indeed, the genetic constitution of the Pœciliidæ is of this XX, XY type, then it follows as a corollary that in the male, which is the heterozygous sex, approximately equal numbers of male- and of female-determining gametes are produced. Assuming that no differential chance-of-fertilization exists we would infer that in the young there would be nearly equal numbers of males and females. This is exactly what we do find under controlled conditions. If then in the adult there is a pronounced excess of females it would appear that this excess must be the end-result of a differential death-rate of the sexes.

A Differential Death-Rate in Gambusia.—The males are much less resistant to harmful environmental factors than the females, and hence have a lower survival value. This is shown by the results of several lines of experiment and observation.

In those cases where quantitative studies have been made with analyzed factors, the males do not survive as well as the females. Thus in the writer's experiments, it was found that high temperatures, high H-ion concentration, oxygen-deficiency, and concentrations of KCN kill the males much more readily than the females. For example, in a collection of 283 young *Gambusia* which was killed in hot water, practically all the fish that died first were males. Bellamy (*in lit.*) states that in his experiments with other Pœciliids with high temperatures, oxygen deficiency KCN, etc., he obtained results that are "in complete agreement" with the writer's contention that "males are more susceptible to 'difficult' conditions than the females."

Unanalyzed deleterious influences in the environment also bring about a lethal selection to which the males succumb more readily than the females. The writer showed (Geiser, '21a), for example, that male *Gambusia* were less resistant than females to disturbances incidental to shipment, during both cold and warm weather. Thus, in cold-weather shipments the male death-rate was one and one half times the female death-rate, and in warm weather shipments, two and one half times the female death-rate. This latter result, also, was obtained when the females were heavily gravid. In aquarium catastrophes, such as epidemics of *Ichthyriophthirius*- and *Saprolegnia*-infestations, the males suffer much more severely than the females. This experience of the writer is confirmed by that of European aquarists generally. In catastrophes of unknown cause, the same holds. Thus, to record one example out of many recorded in my note book: "On 16 November, 1921, in aquarium containing 94♀, 20♂, 16 dead♀ and 14 dead ♂ were found. No cause was ascertained. No fungi. Life-conditions apparently excellent. Death-rate for males 800-, and for females 148.8- per thousand, i.e., 5 3/8 to 1."

The greater ability of the females to survive is moreover evidenced in the proportions of males and females which in the writer's aquaria survive the winter. Thus, e.g., in October, 1921, approximately 100 *Gambusia*, fairly equally divided as to sex, were left outdoors in a somewhat protected concrete aquarium to pass the winter: on April 10, 1922 all the survivors, 32 in number, were recovered. Of these, only one was a male. It is consequently evident that the females were more resistant to the weeding-out process than the males, for only 40 per cent. of the females, as compared with nearly 100 per cent. of the males, had died.

There is still another way in which the numbers of males in a given lot of *Gambusia* are reduced. The males are smaller and hence are more liable to be devoured by small predaceous fish than the much larger females. Gravid female *Gambusia* in aquaria, also, attack and frequently kill the males. Records kept of the sex of dead fish taken from the aquaria show over ten

times as many males as females. This fact lends support to Carbonnier's ('66) contention (Darwin, '75, p. 335) that the males suffer from their small size since they are liable to be devoured by females of their own species.

It is thus apparent that in *Gambusia* there is a differential death-rate, and that its operation explains the excess of females found in adult populations.

Elsewhere the writer (Geiser, '23a, '24) has shown that such a differential death-rate obtains in various fishes, crustacea, insects, for man, and for mammals other than man. Among the fishes besides those already mentioned it has been quite clearly demonstrated in the European Plaice, the Canadian Plaice, Witch, British Salmon, Smelt, and Dogfish, and the Japanese Sweetfish or Ayu. The reader is referred to these papers for the evidence, which conclusively demonstrates that in many diverse groups of animals the male is less viable than the female.

IV. CONCLUSIONS.

1. Field collections of *Gambusia* almost invariably possess a great preponderance of females.
2. These sex-ratios vary with the different seasons of the year.
3. Studies on the spermatogenesis of *Gambusia* fail to reveal any unusual distribution of the chromosomes which would explain the atypical sex-ratios found.
4. Experiments with *Gambusia* raised in aquaria show the proportions of sexes at birth to be approximately equal.
5. The males have a higher death-rate than the females, thus causing the atypical sex-ratios found in the adult populations.

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EXPLANATION OF THE FIGURES.

(All figures were drawn at table level with the aid of a camera-lucida, using a 1.8 Bausch & Lomb Fluorite oil immersion with a Zeiss No. 12 compensating ocular at a tube-length of 160 mm. The drawings thus obtained were enlarged 1.75 diameters with a pantagraph. These final drawings were reproduced without reduction.

PLATE I.

FIGS. 1-2. Dividing spermatogonia from the peripheral portion of the testis.

FIGS. 3-5. Spermatogonia in multiplication-divisions in the cyst.

FIGS. 6-7. Stages later than the preceding, showing reconstitution of the nucleus after spermatogonial divisions.

FIGS. 8-10. Final spermatogonia.

FIGS. 11-16. Leptotene stages.

FIG. 18. Pachytene.

FIG. 19. Cell in the pachytene stage, cut transversely to the chromatin threads.

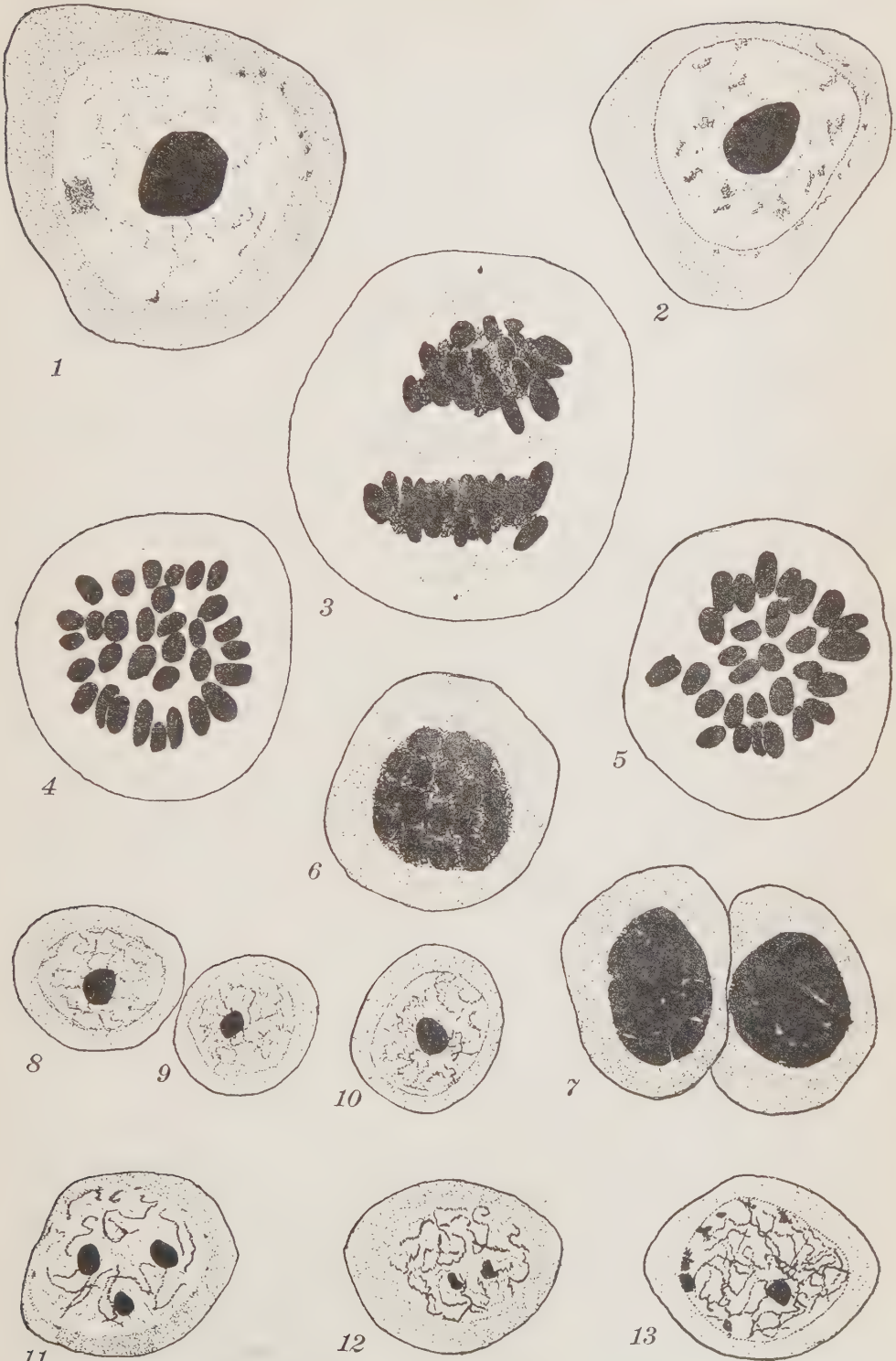


PLATE II.

FIGS. 20-21. Tetrads.

FIG. 22. Diakinesis.

FIG. 23. Tetrads grouping to form an equatorial plate.

FIGS. 24-25. First-spermatocyte divisions, viewed from the side.

FIG. 26. Metaphase plate of second-spermatocyte division.

FIG. 27. Fusion of chromosomes after completion of the second-spermatocyte division.

FIGS. 28-30. Stages in the reconstitution of the spermatid nucleus.

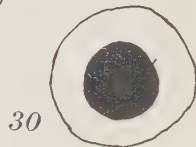
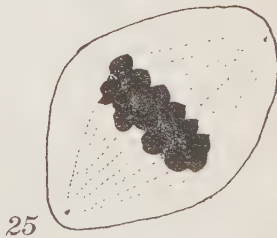
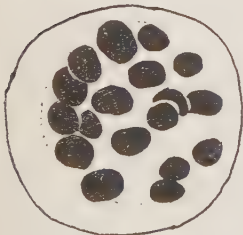
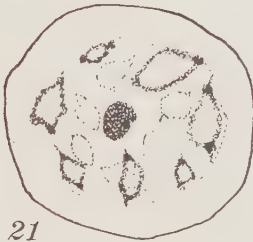
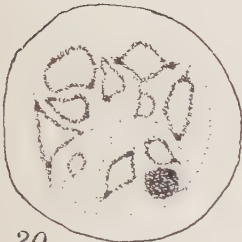
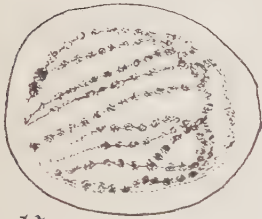
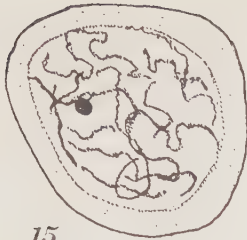


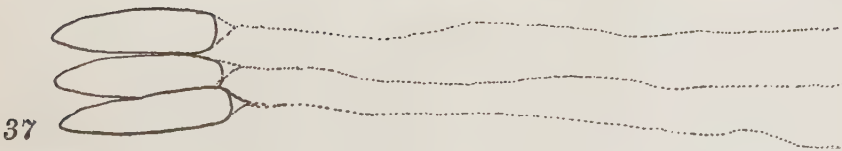
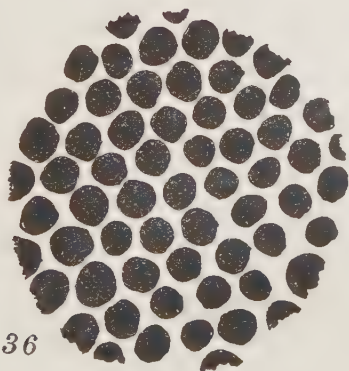
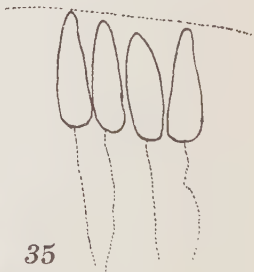
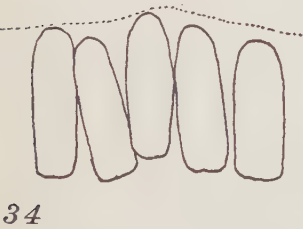
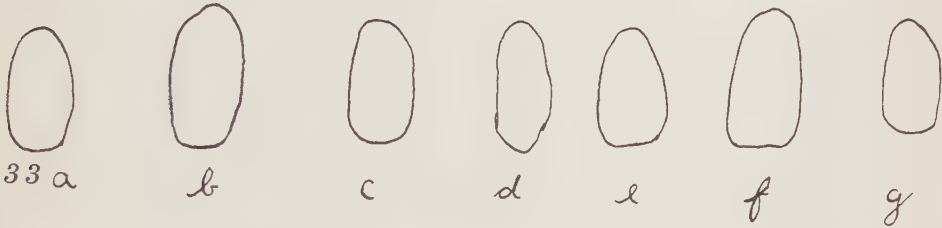
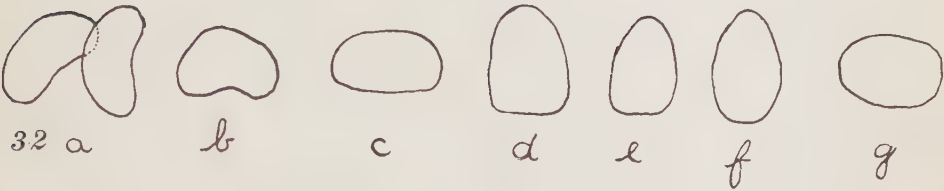
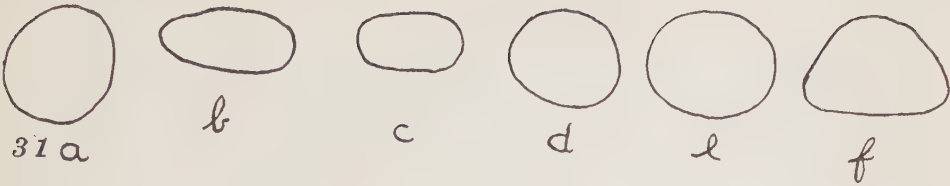
PLATE III.

FIGS. 31-33. Outline drawings showing the forms assumed by the chromatin in the development of the head of the spermatozoon.

FIGS. 34-35. Outline drawings showing the developing spermatozoa arranging themselves at the periphery of the spermatozeugma that is forming.

FIG. 36. Tangential section of the wall of a spermatozeugma, showing the closely-packed arrangement of the spermatozoa in the spermatozeugma.

FIG. 37. Spermatozoa from a nearly-ripe spermatozeugma.



VITA

Samuel Wood Geiser was born in Independence, Iowa, June 11, 1890. After graduation from the public schools of Independence, he matriculated in Upper Iowa University, Fayette, Iowa. From this college he in 1914 obtained his B.A., and in 1919 the degree M.A., *honoris causâ*. From 1914 to 1916 he was Professor of Biology in Guilford College, North Carolina; in 1916-17, Supervisor of Biology and Agriculture in the Independence (Iowa) Schools; from 1917-19, Professor of Biology in Upper Iowa University. In 1919 he commenced graduate study in Zoology in the Johns Hopkins University, taking Plant Physiology and Paleontology as first and second subordinates, respectively. From 1919-21 he served as Research Assistant and Assistant in laboratory instruction to Professor S. O. Mast. In 1921-22, he held the Adam T. Bruce Fellowship in Zoology in the Johns Hopkins University. In 1922 the degree of Doctor of Philosophy was conferred on him by the Johns Hopkins University.

